



# Update on testing the new beta-lactam-inhibitor combinations vs Gram-negative bacteria

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# New BL-BLI agents

Agent	Breakpoints for
Ceftazidime-avibactam	<i>Enterobacterales</i> <i>P. aeruginosa</i>
Ceftolozane-tazobactam	<i>Enterobacterales</i> <i>P. aeruginosa</i>
Imipenem-relebactam	<i>Enterobacterales</i> <i>P. aeruginosa</i> <i>Acinetobacter</i> spp.(with Note)*
Meropenem-vaborbactam	<i>Enterobacterales</i> <i>P. aeruginosa</i> (Note for <i>Acinetobacter</i> *)

\* The beta-lactamases produced by *Acinetobacter* spp. either do not modify the parent carbapenem or are not inhibited by the inhibitor. Therefore the addition of the beta-lactamase inhibitor does not add clinical benefit.

**TO BE CHANGED!**

# Quality control of BL-BLI combinations

## **QC recommendations for MIC determination and disk diffusion (EUCAST QC Tables v 13.1, 2023):**

- The active component is checked with a susceptible QC strain.
- Specific  $\beta$ -lactamase-producing strains are recommended to check the inhibitor component of  $\beta$ -lactam- $\beta$ -lactamase inhibitor combinations.
- Both tests should be part of the routine QC.

[http://www.eucast.org/ast\\_of\\_bacteria/qc\\_tables/](http://www.eucast.org/ast_of_bacteria/qc_tables/)

# QC recommendations for active component

## Example *Enterobacterales*

### *Escherichia coli* ATCC 25922

(NCTC 12241, CIP 76.24, DSM 1103, CCUG 17620, CECT 434)

Antimicrobial agent	MIC (mg/L)		Disk content (µg)	Inhibition zone diameter (mm)	
	Target	Range		Target	Range
Ceftazidime-avibactam	0.125-0.25	0.06-0.5	10-4	<b>27</b>	<b>24-30</b>
Ceftolozane-tazobactam	0.25	0.125-0.5	30-10	28	24-32
Imipenem-relebactam	0.125-0.25	0.06-0.5	10-25	30	27-33
Meropenem-vaborbactam	0.016-0.03	0.008-0.06	20-10	34	31-37

### Fixed concentration of the inhibitor

- Avibactam 4 mg/L
- Tazobactam 4 mg/L
- Relebactam 4 mg/L
- Vaborbactam 8 mg/L

# QC recommendations for the inhibitor

## *Escherichia coli* ATCC 35218

(NCTC 11954, CIP 102181, DSM 5923, CCUG 30600, CECT 943)

TEM-1  $\beta$ -lactamase-producing strain (non-ESBL)

Antimicrobial agent	MIC (mg/L)		Disk content ( $\mu$ g)	Inhibition zone diameter (mm)	
	Target	Range		Target	Range
Ceftolozane-tazobactam	0.125	0.06-0.25	30-10	28	25-31

## *Klebsiella pneumoniae* ATCC 700603\*

(NCTC 13368, CCUG 45421, CECT 7787)

SHV-18 ESBL producer

\* Two colony types are normally observed for this strain and should be included when subculturing and testing the strain.

Antimicrobial agent	MIC (mg/L)		Disk content ( $\mu$ g)	Inhibition zone diameter (mm)	
	Target	Range		Target	Range
Ceftazidime-avibactam	0.5-1	0.25-2	10-4	<b>21</b>	<b>18-24</b>
Ceftolozane-tazobactam	1	0.5-2	30-10	21	17-25

## *Klebsiella pneumoniae* ATCC BAA-2814

KPC-3, SHV-11 and TEM-1

Antimicrobial agent	MIC (mg/L)		Disk content ( $\mu$ g)	Inhibition zone diameter (mm)	
	Target	Range		Target	Range
Imipenem-relebactam	0.125-0.25	0.06-0.5	10-25	25	22-28
Meropenem-vaborbactam	0.25	0.125-0.5	20-10	<b>18</b>	<b>15-21</b>

# Example QC BMD

<i>E. coli</i> ATCC 25922	
Ceftazidime-avibactam	Ceftolozane-tazobactam
≤0.12	0.25
≤0.12	0.5
≤0.12	0.25
≤0.12	0.25
≤0.12	0.25
0.25	0.25
≤0.12	0.25
≤0.12	0.5
≤0.12	0.5
≤0.12	0.5
≤0.12	0.5
≤0.12	0.25
≤0.12	0.5
≤0.12	0.5
≤0.12	0.25
≤0.12	0.25

<i>P. aeruginosa</i> ATCC 27853	
Ceftazidime-avibactam	Ceftolozane-tazobactam
2	1
2	0.5
2	0.5
1	0.5
1	0.5
2	1
2	1
2	1
2	1
2	1
1	1
2	0.5
2	1
1	1
2	1

<i>K. pneumoniae</i> ATCC 700603	
Ceftazidime-avibactam	Ceftolozane-tazobactam
0.5	2
0.5	2
0.5	1
0.5	1
0.5	1
0.5	1
0.5	1
0.5	1
0.5	1
1	1
0.5	2
0.5	1
0.5	2
0.5	2
0.5	2
0.5	1

Target  
Range

0.125-0.25

0.25

0.06-0.5

0.125-0.5

1-2

0.5

0.5-4

0.25-1

0.5-1

1

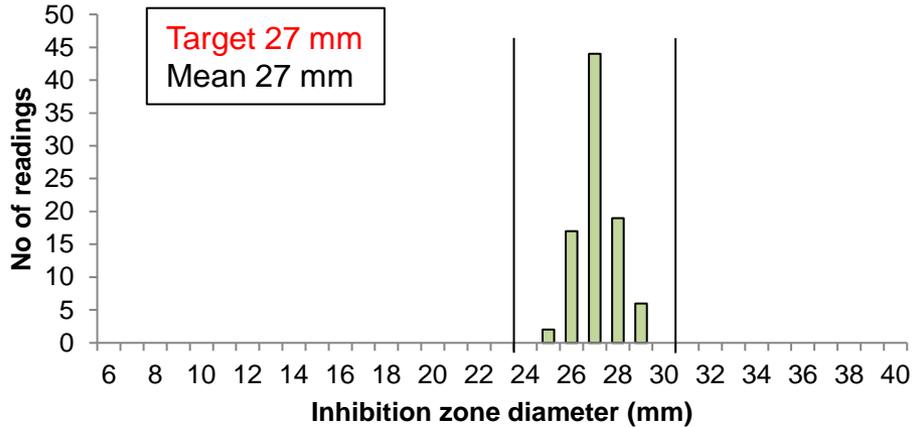
0.25-2

0.5-2

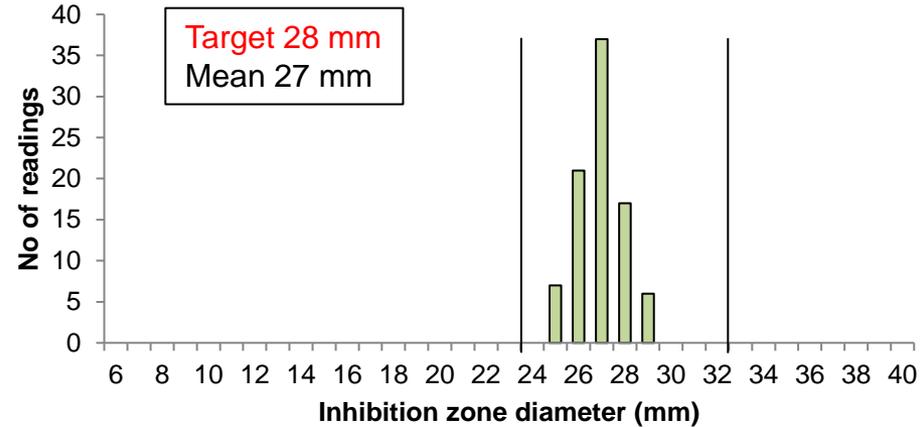
On target
On upper limit
On lower limit
Out of range

# Example QC disk diffusion

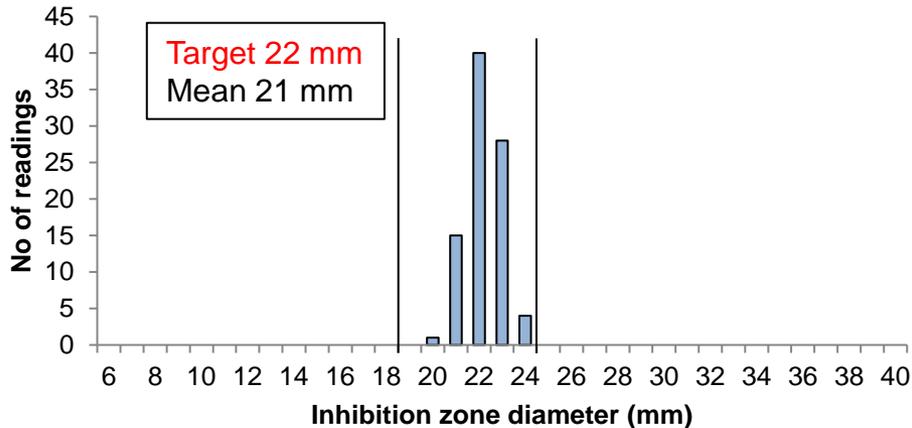
*E. coli* ATCC 25922  
Ceftazidime-avibactam 10-4 µg



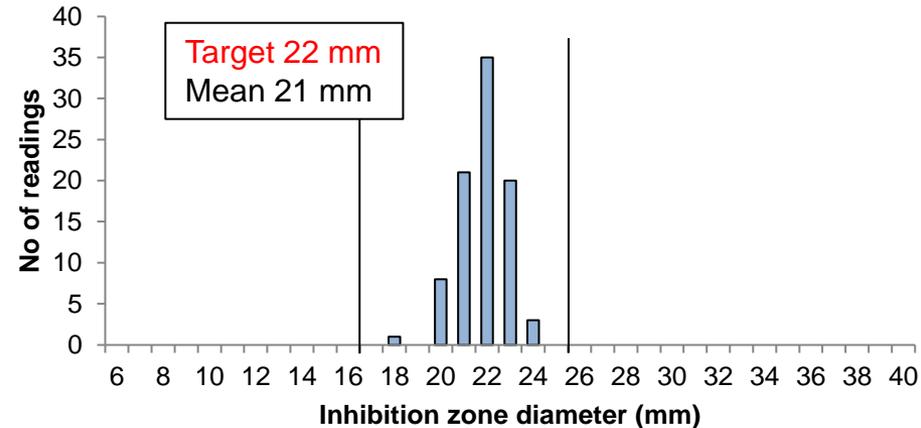
*E. coli* ATCC 25922  
Ceftolozane-tazobactam 30-10 µg



*K. pneumoniae* ATCC 700603  
Ceftazidime-avibactam 10-4 µg

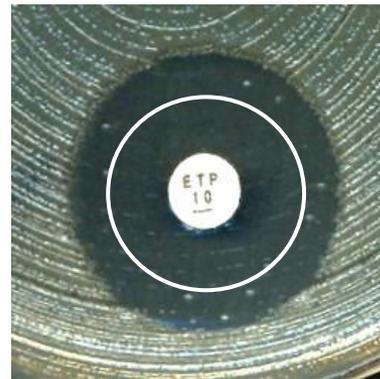
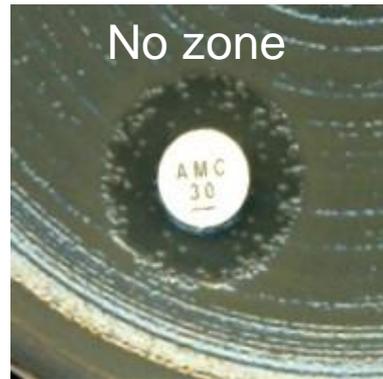


*K. pneumoniae* ATCC 700603  
Ceftolozane-tazobactam 30-10 µg



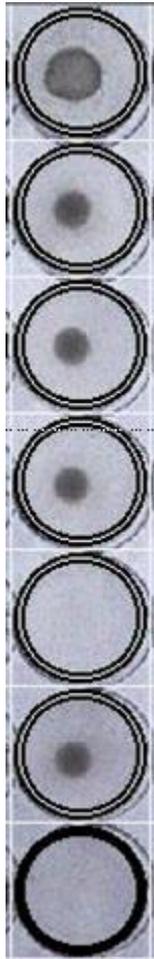
# Testing issues DD: Colonies within zone

- In case of distinct colonies within zones, check for purity and repeat the test if necessary.
- If cultures are pure, colonies within zones should be taken into account when measuring the diameter.



Reading of zones with colonies within the zone.

# Testing issues BMD: Skipped wells



0.5

1

2

4

8

16

32

## Example

*Enterobacteriales* and ceftazidime-avibactam  
S $\leq$ 8, R $>$ 8 mg/L

← 8 mg/L, susceptible?

← 32 mg/L, resistant?

- **Retest!**
- Compare with disk diffusion results
- Report the highest MIC

# Testing issues BMD: Haze in wells

- Haze or turbidity without a pellet is often seen for *Pseudomonas* spp. and *Acinetobacter* spp. This should be regarded as growth and the endpoint read at the first well with complete inhibition (clear broth).



- If light haze
  - Compare with growth control
  - Tilt the plate (preferably by using a mirror)
  - Compare with disk diffusion results
  - Ask a colleague for a second opinion



# Gradient tests for testing the new BL-BLI combinations

- No EUCAST evaluation performed
- Check the EUCAST warning website for current warnings
  - The fact that there is no warning on the EUCAST website does not mean that there might not be problems with these products
- If you decide to use gradient tests, evaluate with QC strains (all available strains with QC criteria) and isolates/strains with elevated MICs

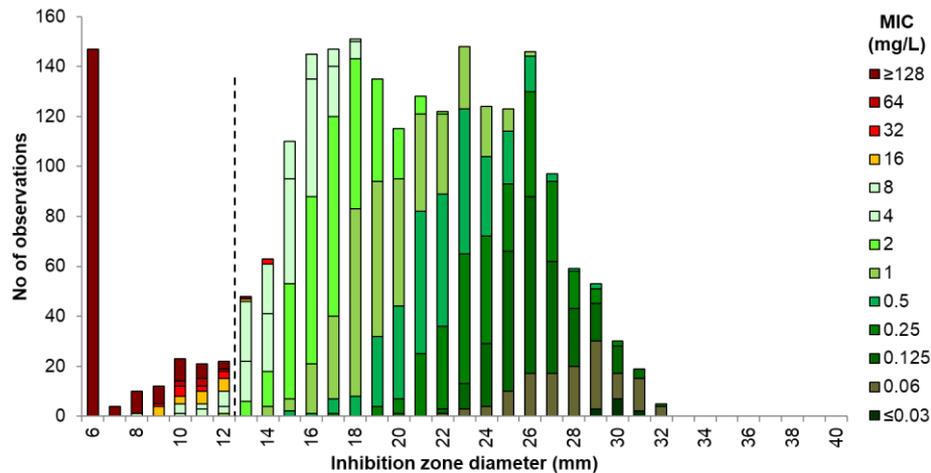
<https://www.eucast.org/ast-of-bacteria/warnings>

# EUCAST recommendations

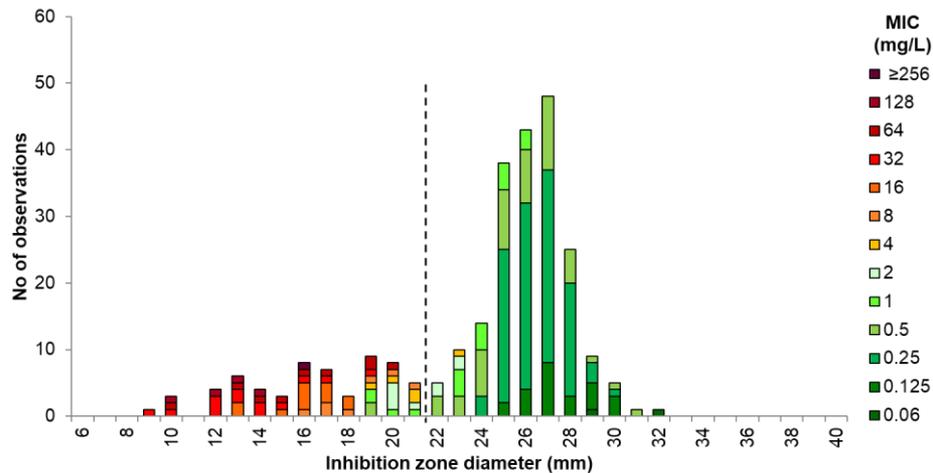
- Use disk diffusion or broth microdilution for AST of BL-BLI combinations
  - Perform QC with both a susceptible and a  $\beta$ -lactamase producing strain
- Disk diffusion
  - Make sure that mean zone diameters for QC strains are on target  $\pm$  1 mm
- Broth microdilution
  - Commercial products available
    - Several QC strains are usually needed to avoid truncated MIC values
  - EDL performs BMD on requests (see <https://mikrobiologi.org/referenslaboratorium>)

# Zone diameter breakpoints and ATUs

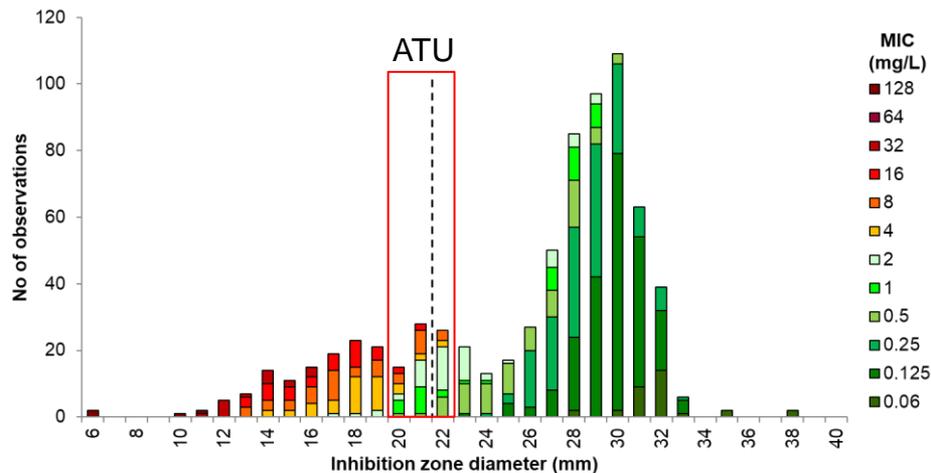
**Ceftazidime-avibactam 10-4 µg vs. MIC**  
*Enterobacterales*, 293 isolates (2207 correlates)



**Ceftolozane-tazobactam 30-10 µg vs. MIC**  
*Enterobacterales*, 260 isolates



**Imipenem-relebactam 10-25 µg vs. MIC**  
*Enterobacterales*, 180 isolates (720 correlates)



**Meropenem-vaborbactam 20-10 µg vs. MIC**  
*Enterobacterales*, 104 isolates (412 correlates)

